with markedly reduced risk for GVHD in lethally irradiated mice (Ophir *et al* Blood 2010). We now show that these Tcm can also enhance engraftment following sublethal TBI (5.5Gy). Thus, while all 13 BALB/c recipients of 10^7 Nude-B6 BM cells rejected the graft by day 30, 6/7 mice receiving also $5x10^6$ (B6xBALB/c)F1 Tcm exhibited near full chimerism (81±11%, > 6 months follow up). Importantly, chimerism was associated with successful engraftment of donor skin (> 3 months follow up) in 4/5 chimeras, while 3rd-party unrelated skin was promptly rejected. When allogeneic B6 Tcm were used, 9/9 mice displayed full donor chimerism and 5/7 maintained donor skin for over 3 months, without any GVHD symptoms (weight and overall appearance were the same as that of control mice). Considering that RIC may be associated with increased relapse rate, it was of interest to evaluate the GVL reactivity of Tcm. We previously showed that anti 3rd-party human CTLs eliminate B-CLL and other lymphoma types by unique TCR independent mechanism. This killing initiates with ICAM1-LFA1 adhesion, followed by slow apoptosis induced by interaction of CD8 on the CTL with MHC-I on the tumor cell. Initially, we verified that the anti 3rd-party Tcm can also kill murine A20 lymphoma cells in-vitro (34.8±12.1% after 16hrs of co-incubation). This killing was mediated by apoptosis (AnnexinV staining of A20 cells increased from 5.2±2%, to 14.8±4.5%, $p < 0.05$). Next, using a model simulating minimal residual disease, we followed luciferase expressing A20 cells in-vivo, and studied the effect of adding Tcm to BMT. Thus, lethally irradiated BALB/c mice were transplanted with $3x10^6$ allogeneic Nude B6 BM and 5000 A20 cells. While all 8 untreated mice died from tumor overload by day 28, treatment with $5x10^6$ donor type Tcms one day post BMT led to tumor elimination and overall survival of 100% (7/7) 100 days post BMT, without any manifestation of GVHD.

Collectively, our data suggest that anti 3rd-party Tcm can provide a 'double supportive effect' by promoting BM engraftment under RIC, and inducing GVL reactivity, without causing GVHD. Such cell therapy could be highly attractive for patients with B cell malignancies who might not tolerate aggressive conditioning.

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COMPARATIVE ANALYSIS OF OUTCOMES OF ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FROM RELATED AND UNRELATED DONORS FOR ACUTE MYELOID LEUKEMIA

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This study compared the results of allogeneic peripheral blood stem cell transplantation (PBSCT) from unrelated and related donors in 142 consecutive patients with AML. Among these patients, 101 (71.1%) received an HLA-matched related PBSCT and 41 (28.9%) an HLA-matched unrelated PBSCT. High-risk AML was defined as the presence of adverse cytogenetics or failure to achieve CR. Among the 41 (28.9%) patients assigned to the high-risk group, 35 (85.4%) patients had failed to achieve CR and 7 patients (17.1%) had adverse cytogenetics. The cumulative incidence of acute graft-versus-host disease (GVHD) was 37.6% in the related PBSCT group and 53.7% in the unrelated PBSCT group. The cumulative incidence of extensive chronic GVHD was higher in the unrelated PBSCT group (19.5%) than in the related PBSCT group (8.9%). The overall survival (OS) rate at 4 years was 62.4±5.4% and 53.8±1.2% and the cumulative incidence of relapse was 24.8% and 12.2% in the related and unrelated PBSCT groups, respectively. In a multivariate analysis, unrelated PBSCT was identified as a risk factor for the development of extensive chronic GVHD (hazard ratio = 3.019, *P*-value = 0.027). Among the factors examined, unfavorable cytogenetics and the disease status at the time of transplantation were found to be related with overall survival. In the case of high-risk AML, the survival rate and relapse incidence were significantly better in the matched unrelated PBSCT group (P-value = 0.047, P-value = 0.039, respectively). In conclusion, the alloPBSCT outcomes for AML were comparable in the matched related and matched unrelated groups. Nonetheless, for high-risk AML patients, matched unrelated PBSCT was found to be preferable to matched related PBSCT.

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STAT3 PROMOTES BOTH NATURAL AND INDUCIBLE T REGULATORY CELL PLASTICITY DURING MURINE ACUTE GVHD

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Adoptive cell transfer of FoxP3+ regulatory T cells (Tregs) has been proposed as a novel therapy for prevention of acute GVHD. Such therapy may theoretically be limited by conversion of Tregs into pathological effector T cells due to STAT3-activating inflammatory cytokines, including IL-6, IL-21, and IL-23. To better understand the role of STAT3 in Treg cell plasticity during acute GVHD, we used a B6 ⇒ BALB/c transplantation model. Donor cell populations were obtained from either, STAT3 fl/fl Foxp3-GFP (WT) or CD4-Cre, STAT3 fl/fl-Foxp3 GFP (KO). Transfer of T cell-replete, WT inocula uniformly resulted in lethality by day 14 post-BMT; in contrast, T cell-replete, STAT3-KO recipients had 100% post-BMT survival ($p < 0.001$). Relative to WT recipients, STAT3-KO recipients also had less GVHD by histology assessment ($p < 0.05$) and had increased numbers of splenic FoxP3+ T cells post-BMT ($p = 0.005$); in contrast, WT recipients had increased IL-17 and IFN-γ secreting T cells post-BMT (each, $p < 0.01$). The increased number of post-BMT FoxP3+ cells in STAT3-KO recipients may have been due to: (1) increase in "induced" Tregs from the naïve T cell pool; and (2) decrease in loss of "natural" (n)Tregs. Purified, naïve, FoxP3 negative T cells from WT or STAT3KO donors were transferred to evaluate the first possibility: after allogeneic BMT, FoxP3 induction was greatly increased in STAT3-KO recipients relative to WT recipients ($p < 0.01$). And, to address the second possibility, purified WT and STAT3-KO FoxP3+ Tregs were co-infused with conventional donor T cells: relative to recipients of WT nTregs, recipients of STAT3-KO nTregs had greatly increased post-BMT numbers of FoxP3 post-BMT ($p < 0.05$). As such, the in vivo pool of Treg cells after allogeneic BMT is controlled to a great extent by STAT3 signaling, which influences both natural and induced Treg pathways.

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IMPACT OF REDUCTION IN GVHD-RELATED MORTALITY FOR RECENT IMPROVEMENT OF NON-RELAPSE MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Purpose: We retrospectively assessed changes in the incidence and causes of non-relapse mortality (NRM) over the last 12 years in allo-HCT patients.

Methods: We analyzed a nationwide registry database that includes patients aged 16 years or older with AML or ALL in remission, or low-risk MDS who received allo-HCT from 1997 to 2008. We compared the incidence of NRM and overall survival (OS) after allo-HCT in three consecutive four-year periods (1997-2000, 2001-2004, and 2005-2008) for younger patients (16-49 years), and in the later two periods for older patients (≥ 50 years). Subgroup analyses were performed based on patient age and donor source: HLA-matched/1-Ag mismatched related donor (related donor) versus unrelated BM or CB donor (alternative donor).

Results: A total of 6517 patients with a median age of 40 years were analyzed and the median follow-up was 39 months. A total of 1354, 2298, and 2865 allo-HCT were performed in 1997-2000, 2001-2004, and 2005-2008, respectively. The incidence of NRM was 23% at 3 years after allo-HCT. A subgroup analysis of younger patients who received allo-HCT from a related donor showed that there was no significant change in NRM (12-15%). In younger patients who received allo-HCT from an alternative donor, the

NRM incidence significantly decreased over the three periods (30%, 24%, and 22%, $p < 0.001$) mainly due to a reduced risk of death associated with organ failure (14%, 9%, and 7%, $p = 0.005$) and GVHD (7%, 3%, and 3%, $p < 0.001$), which led to a significantly improved OS (58%, 59%, and 64%, $p = 0.008$). In older patients who received allo-HCT from a related donor, NRM significantly improved in 2005-2008 compared to 2001-2004 (29% vs 18%, $p < 0.001$) due to a reduced risk of death associated with organ failure (11% vs 6%, $p = 0.007$) and GVHD (6% vs 3%, $p = 0.11$). However, due to the increase in the incidence of relapse in 2005-2008, this decreased NRM did not lead to an improvement of OS (51% vs 55%, $p = 0.21$). In older patients who received allo-HCT from an alternative donor, NRM and OS significantly improved in 2005-2008 compared to 2001-2004 (NRM, 43% vs 31%; OS, 40% vs 51%, $p < 0.001$). The reduction in NRM was mainly due to a decrease in death associated with infection (17% vs 11%, $p = 0.007$) and GVHD (7% vs 4%, $p = 0.152$).

Conclusions: NRM and OS have recently improved, especially for allo-HCT from unrelated BM or CB donors. These advances seemed to be due to a reduced risk of death associated with GVHD in both younger and older patients.

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OLDER SIBLING DONORS REMAIN AN EXCELLENT OPTION: OLDER DONOR AGE AND LOWER CD34 INFUSED DO NOT ADVERSELY IMPACT OUTCOMES FOR MATCHED SIBLING PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION

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Intro: For unrelated donor allografts, higher cell doses improve outcomes. Increasingly, older patient age is prompting consideration of older sibling donors. On average, older donors mobilize PBSC less well. We hypothesized that outcomes would be worse after older sibling PBSC allografts as older age adversely impacts PBSC yields.

Methods: We reviewed transplant outcomes from HLA matched or single antigen/allele mismatched siblings harvested between January 2001 and April 2008. Donors underwent uniform PBSC mobilization of G-CSF at 10 mcg/kg/day and apheresis on day 5. We evaluated the influence donor and recipient age as continuous variables. We also explore the allograft dose by CD34 dose infused above the median and the peripheral blood (PB) CD34 on day 5 pre-apheresis (a biologic measure of mobilization capacity of the donor). All diseases and conditioning regimens were included. The majority of patients underwent regimens incorporating campath.

Results: Of the 195 sibling donors who underwent mobilization, 182 pts (93%) were infused with PBSCs from one mobilization attempt. The median donor age was 52 years (range 16-70) and 16.9% were 60 years or older. Median recipient age was 53.2 years. The median CD34 infused was 5.4 x10(6)/kg (range 0.34 to 13.2). The risk of acute II-IV GVHD did not correlate with older donor age (HR = 1.01, P0.49), recipient age (HR = 1.04, 95%CI, 0.99-1.09; P = 0.06), CD34 infused above the median (HR = 1.19, P = 0.12), or higher pre-apheresis PB CD34 (HR = 1.01, P = 0.82). However, only 22 recipients developed aGVHD. Donor age was not associated with relapse free survival (RFS) (HR = 1.06, P = 0.45) or OS (HR = 1.06, P = 0.53). Older recipients, however, had inferior OS (HR = 1.02, P = 0.014) but not RFS (HR = 1.01, P = 0.30). OS was not associated with peak PB pre-apheresis CD34 (P = 0.73) or CD34 infused (P = 0.85). In multivariate analysis of donor age, recipient age, disease status and CD34 infused, higher CD34 infused did not impact OS. While older recipient age demonstrated a strong association with poorer OS (P = .008), older donor age showed a borderline associated with improved OS (PS = .049).

Conclusion: After adjusting for recipient age and disease, lower CD34 infused and older donor age had no detrimental impact on GVHD, relapse, or OS. Absent data showing a benefit of younger unrelated donors, we conclude that medically cleared older sibiling donors should be considered the standard of care when available, at least up to age 70 years.

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ALEMTUZUMAB VS ATG FOR T-CELL DEPLETION IN SIBLING DONOR REDUCED INTENSITY HAEMATOPOIETIC STEM CELL TRANSPLANTATION (RIC HSCT) FOR THE TREATMENT OF ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASTIC SYNDROME

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We report the results of a retrospective analysis on 83 patients who underwent HLA-matched sibling donor RIC HSCT receiving either ATG or alemtuzumab. All patients received a uniform protocol of fludarabine and busulfan. For T-cell depletion/GVHD prophylaxis 48 patients received alemtuzumab(20mg x 5 days IV) followed by cyclosporin A post-transplant. 35 patients received ATG(Thymoglobulin, Genzyme)(total 6mg/kg over 3 days IV) followed by methotrexate/cyclosporine A post-transplant.

Diagnoses included myelodysplastic syndrome (MDS)(n = 34), acute myeloid leukaemia (AML)(n = 40) or MDS/MPD(n = 9). Median recipient age was 56yrs(range 22-72) in both groups. All patients received PBSC with median CD34 cell dose of 5.06 $x10^6$/kg. There were no significant differences between groups with regard to age, disease type, cytogenetic risk, disease stage at transplantation, or CD34 cell dose. Median follow-up (for survivors) was 743 days (range 31-1516) for ATG and 2193 days (range: 39-3595) for alemtuzumab.

Median time to neutrophil (0.5x10^9/L) regeneration was 14 days in both groups. There were 3 primary graft failures (1 ATG, 2 alemtuzumab). Median donor CD3 chimerism to day 100(alemtuzumab v ATG) was 73% v 65% at D30, 74% v 69.5% at D60 and 67% v 44% at D100(p = NS). A higher proportion of patients receiving alemtuzumab required subsequent DLI therapy (68.8 v 31.4% p = 0.001). The main reason for DLI in each group was falling donor chimerism (61 v 57.1%p = NS). The incidence of de novo cGVHD was higher in the ATG arm(25.6 v 8.3% p = 0.06), however this was offset by subsequent development of cGVHD in the alemtuzumab arm due to greater DLI administration. Consequently overall rates of chronic GVHD were similar between alemtuzumab and ATG arms(41.2% v 40%). The 2-year OS in the ATG v alemtuzumab group was 67.8 +/− 8.5% vs 50.3 +/− 7.4%(p = 0.38). DFS was 51 +/− 9.4% in ATG patients, vs 38.3 +/−7.1% in alemtuzumab patients. There was no significant difference in TRM or relapse between the alemtuzumab vs ATG group 27+/−7.5% v 15+/−6.9%, (p = 0.48), and 47.5+/−7.8% vs 43.8 +/− 9.8%.

In summary, our experience indicates that in patients with MDS/AML having sibling donor RIC HSCT, use of ATG results in comparable outcomes when compared with alemtuzumab, with significantly lower need for DLI. While the incidence of GvHD is similar between groups, ATG patients are more likely to develop de novo GvHD when compared with alemtuzumab patients who develop delayed GvHD following DLI.

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SILICA-INDUCED IMPAIRMENT OF MACROPHAGE FUNCTION REDUCES GVHD SEVERITY AND IMPROVES SURVIVAL

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Acute graft versus host disease (GVHD) is a major complication of bone marrow transplantation (BMT). The pathogenesis of GVHD is characterized by a cascade of inflammatory cytokines and effector molecules that maintain a proinflammatory milieu capable of continuously activating immune cells leading to tissue injury. Several studies have demonstrated a role for macrophages in secreting these proinflammatory mediators during GVHD progression. Silica decreases the phagocytic capacity of macrophages and alters their capacity to activate T cells by switching from a Th1 to a Th2 response. Therefore, we investigate whether altering macrophage function by *in vivo* administration of silica would result in alleviation of GVHD symptoms and improved survival. Mice were lethally irradiated and transplanted with with $5x10^6$ allogeneic T-cell depleted bone marrow cells (TCD-BM) and $7.5x10^5$ allogeneic conventional T cells (Tcon) to induce GVHD. A significant increase in median survival